circio

Building next generation RNA therapeutics

Investor webcast

11 October 2023

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1

circRNA introduction

2. circVec Data
3. Rare disease data
4. Summary & Next steps

Presenters today - The discoverers of circRNA

Dr Thomas B Hansen

Dr Erik D Wiklund

nature — 6,373 citations

Published: 27 February 2013

Natural RNA circles function as efficient microRNA sponges

Thomas B. Hansen, Trine I. Jensen, Bettina H. Clausen, Jesper B. Bramsen, Bente Finsen, Christian K. Damgaard & Jørgen Kjems

THE EMBO JOURNAL — EMBOpress — 30 September 2011 — 922 citations

CURRENT ISSUE ABOUT INFORMATION ARCHIVE ALERTS SUBMIT

miRNA-dependent gene silencing involving Ago2-mediated cleavage of a circular antisense RNA

Thomas B Hansen, Erik D Wiklund, Jesper B Bramsen, Sune B Villadsen, Aaron L Statham, Susan J Clark, Jørgen Kjems

nature reviews genetics — 2,291 citations

Review Article | Published: 08 August 2019

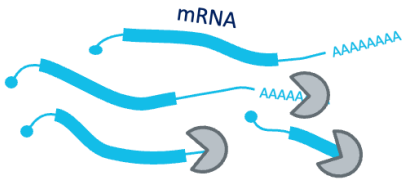
The biogenesis, biology and characterization of circular RNAs

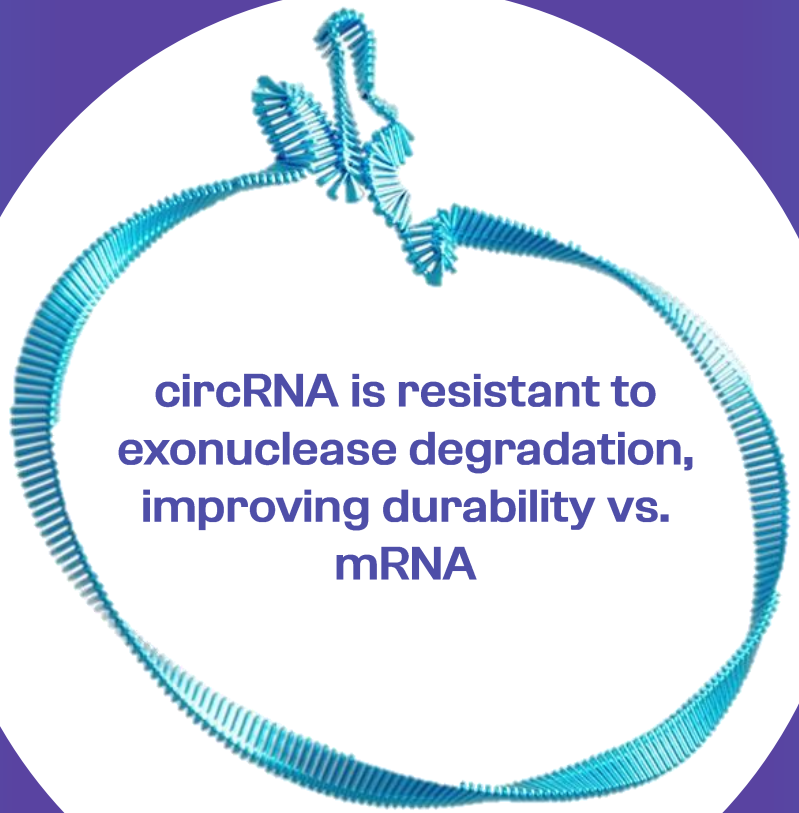
Lasse S. Kristensen, Maria S. Andersen, Lotte V. W. Stagsted, Karoline K. Ebbesen, Thomas B. Hansen & Jørgen Kjems

circio

circRNA provides a toolbox to create a novel class of medicines

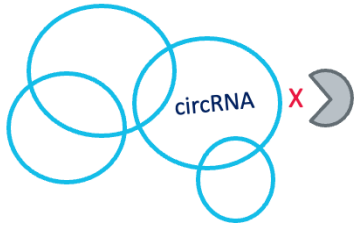
Extended RNA durability





circRNA is resistant to exonuclease degradation, improving durability vs. mRNA

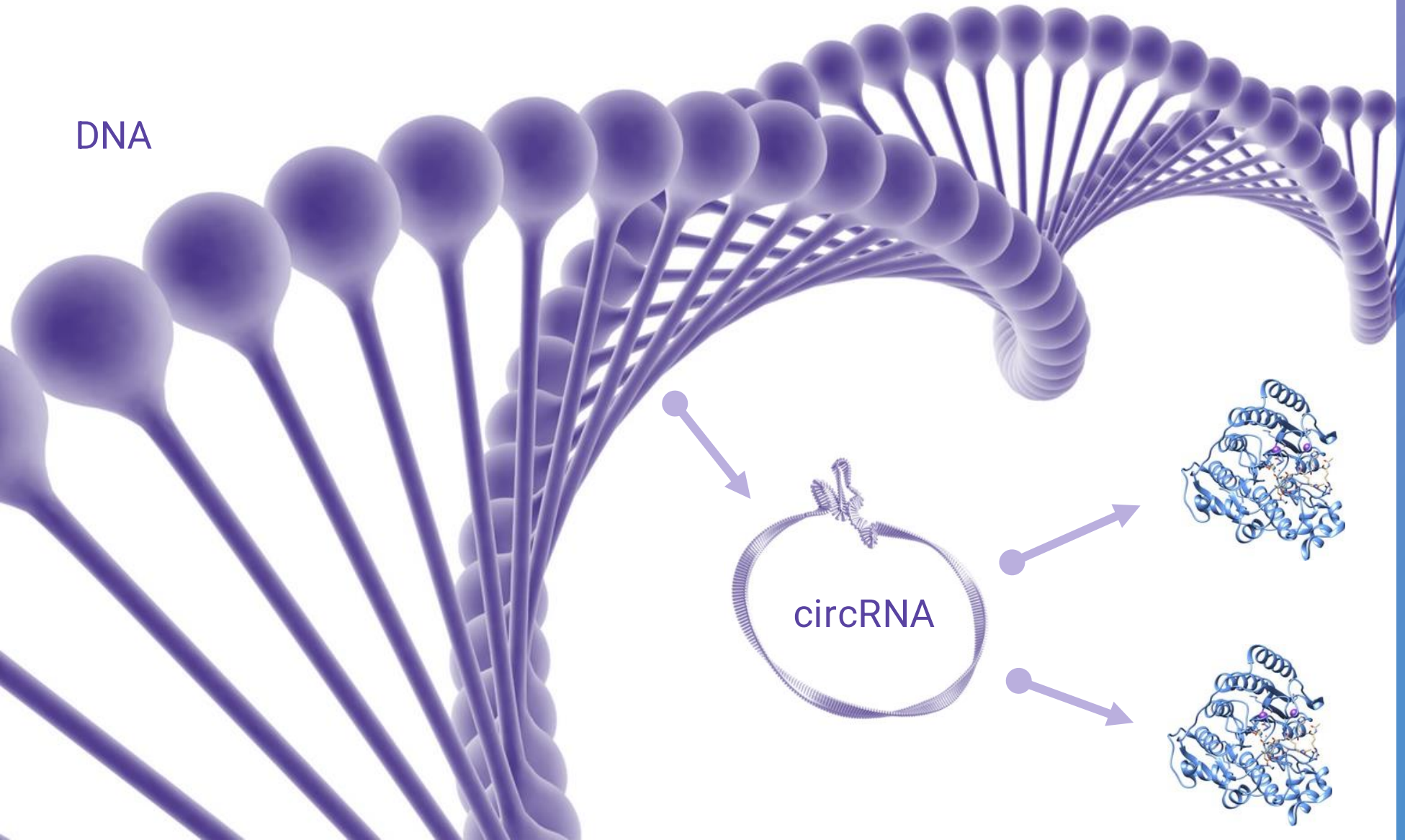
Enhanced protein expression



microRNA sponging

Regulatory functionality

circVec – Circio`s proprietary vector system for intra-cellular protein expression



circVec
DNA or viral vector

Inject

circRNA biogenesis

Intra-cellular protein expression

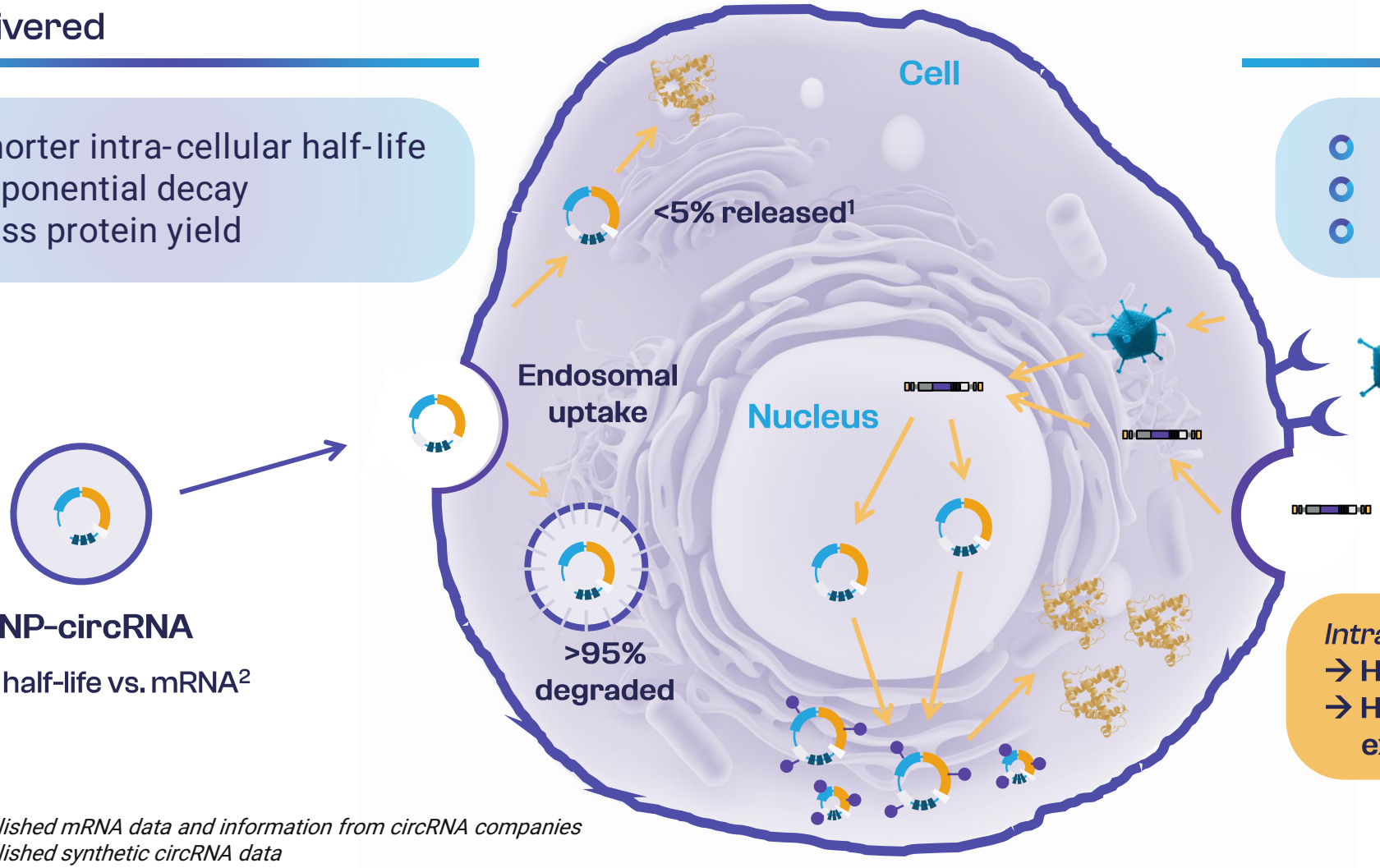
Intra-cellularly generated circRNAs have extended durability vs. synthetic LNP-packaged circRNA

Synthetic circRNA,
LNP delivered

- Shorter intra-cellular half-life
- Exponential decay
- Less protein yield

Intra-cellularly generated circRNA,
vector delivered

- Longer intracellular half-life
- High steady-state expression
- Higher protein yield



LNP-circRNA

3-5x half-life vs. mRNA[2]

circVec
Virus or DNA vector

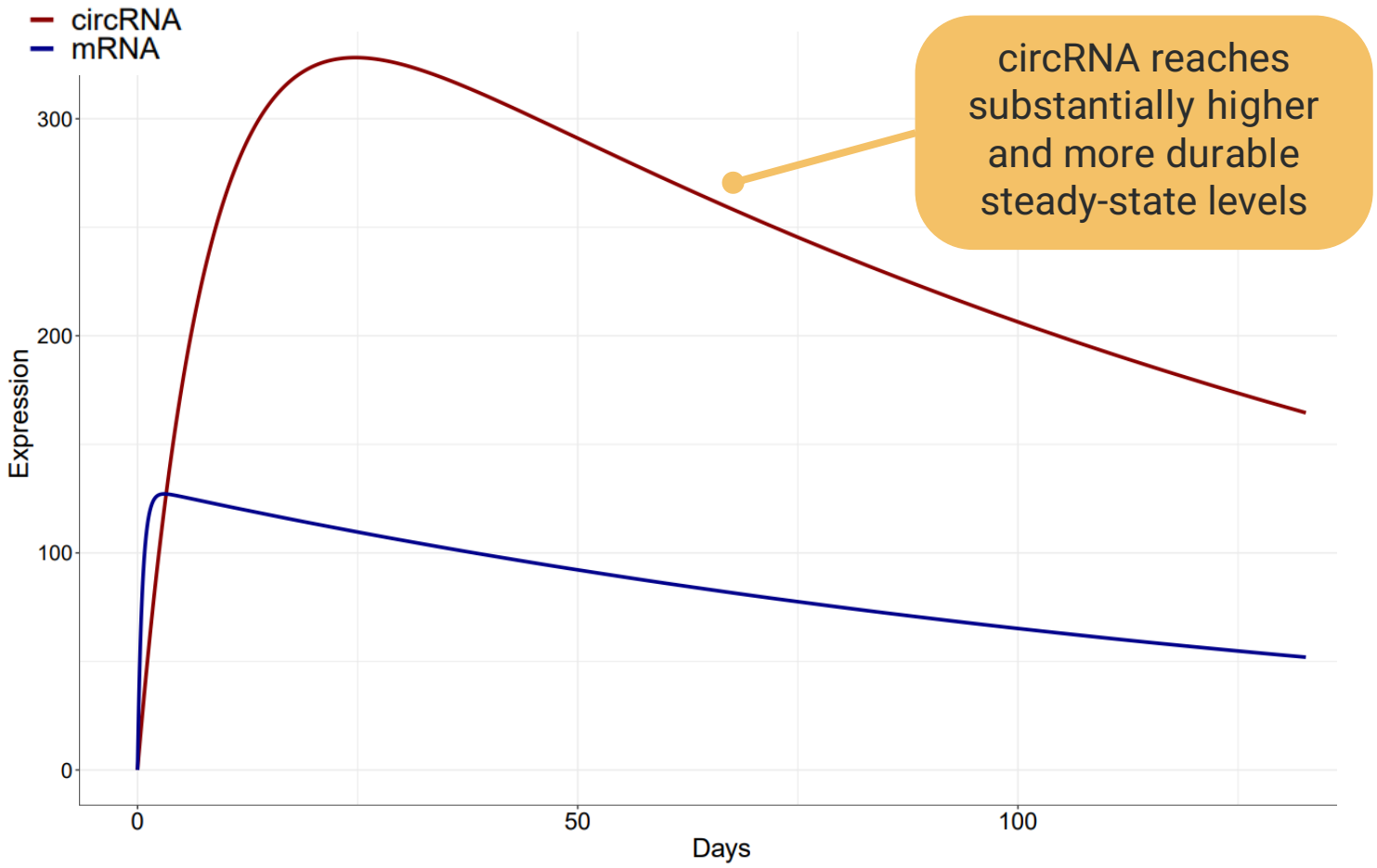
15x half-life vs. mRNA[3]

Intra-cellular circRNA biogenesis:
→ **Higher steady-state circRNA levels**
→ **Higher and longer-lasting protein expression**

(1) Based on published mRNA data and information from circRNA companies
(2) Based on published synthetic circRNA data
(3) Based on Circio's in vitro results

Bioinformatic simulation demonstrating advantage of vector-expressed circRNA vs. mRNA

Temporal vector-based RNA expression dynamics; circRNA vs. mRNA



* Based on circVec experimental data

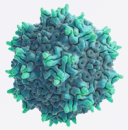
Input assumptions for simulation:

Non-dividing target cells

Vector half-life:	100 days
mRNA production:	10 molecules / hr
mRNA half-life:	9 hrs *
circRNA production:	2 molecules / hr *20% of mRNA rate*
circRNA half-life:	135 hrs * *15x mRNA ½-life*

→ *circRNA translation 3-5x mRNA rate* gives 10-15x peak protein expression*

Both viral and synthetic DNA vector formats are being tested for therapeutic applications

	Viral		Synthetic DNA	
	 AAV	 Adenovirus	DNA format 1 	DNA format 2
Application	• Gene therapy	• Vaccines • Oncology	• Gene therapy • Vaccines	• Gene therapy
Aim	• Improved expression and reduced dosing vs. mRNA AAV	• Single-dose vaccine • Therapeutic protein delivery to tumors	• Enable repeat-dosing for gene therapy • Enhanced nuclear uptake	• Improved uptake • Reduced immunogenicity
	Advantage: efficient delivery of genetic material *Challenge: Repeat dosing and immune response*		*Advantage: repeat dosing and manufacturing* *Challenge: Nuclear delivery and innate immunity*	

Synthetic DNA vector: Neoregen collaboration

- *Collaboration announced 10 October*
- *Test delivery of circVec DNA vectors using Neoregen´s proprietary peptide chemistry*

Synthetic DNA

DNA format 1	DNA format 2
Gene therapy Vaccines	Gene therapy
Enable repeat-dosing for gene therapy Enhanced nuclear uptake	Improved uptake Reduced immunogenicity

Advantage: repeat dosing and manufacturing
Challenge: Nuclear delivery and innate immunity

circVec offers clear advantages in multiple therapeutic areas, and opens new opportunities for circRNA

Current focus areas

Rare disease

"Remove-and-replace" concept with durability and safety advantages

-

Major long-term potential

Vaccines

Enhanced potency, single dose vaccine concept with simplified administration

-

Early partnering option

Cancer gene therapy

Efficient and durable expression of therapeutic proteins in solid tumors

-

Unique oncology concept

Designed for intra-cellular circRNA supply, durable protein expression and targeted regulatory functionality

The circVec "killer-app" gene therapy proof-of-concept

vector

Standard mRNA approach

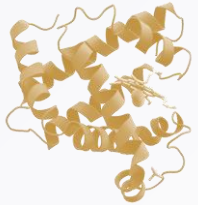
circVec circRNA approach

Shorter durability

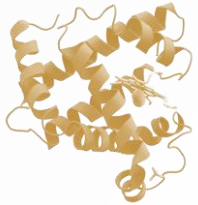
RNA

15x durability
3-5x translation rate

Lower steady-state protein levels



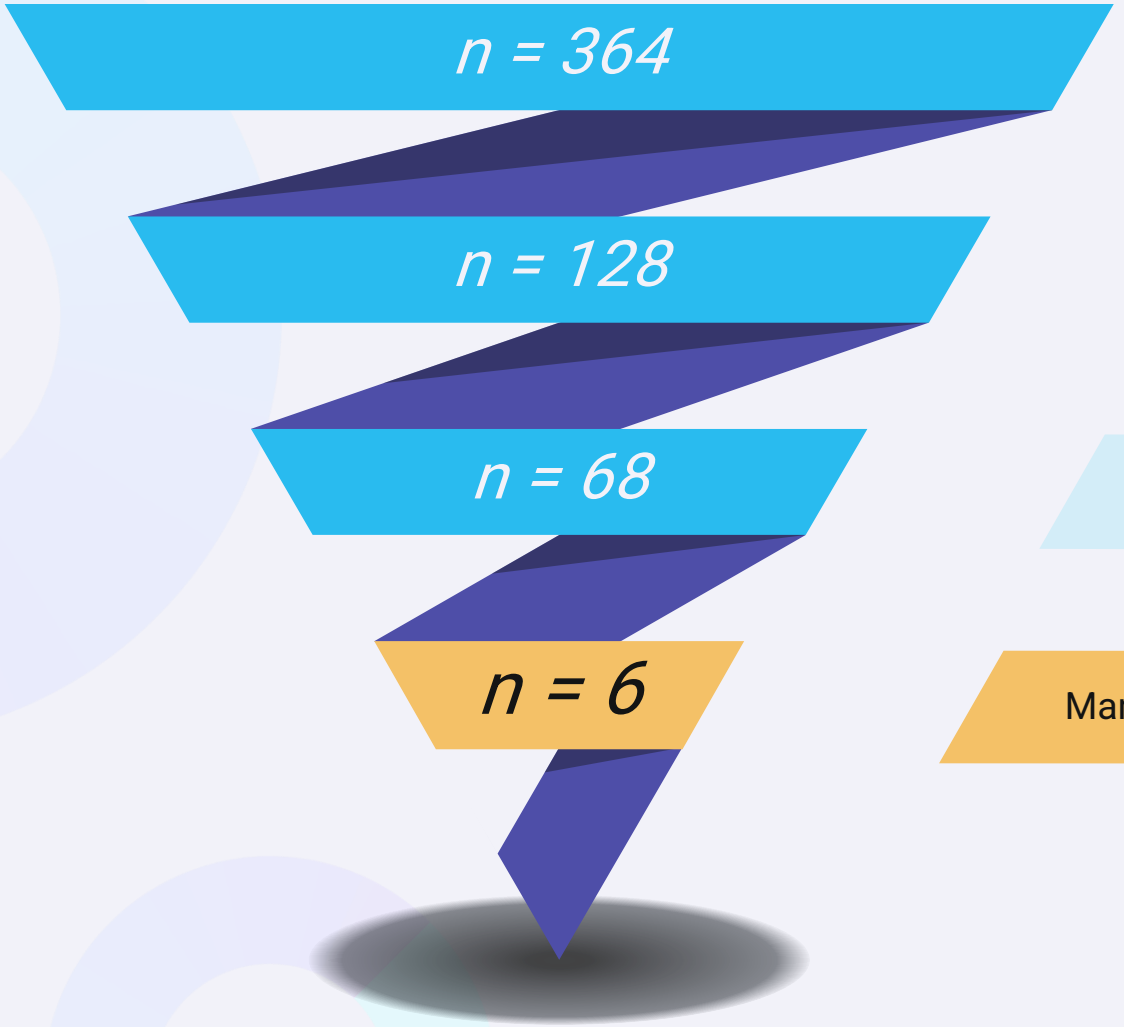
Protein



10-15x maximum protein levels

circVec could enable improved safety, lower dosing and reduced cost for AAV gene therapy

Screening and prioritization ongoing to identify target rare diseases suitable for circVec approach



Initial Screening

- Therapeutic Focus: Rare genetic disorders
- Development Stage: Clinical or Market validation achieved

1st Prioritization Criteria

- Etiology: monogenic disorders only (exclude polygenic disorders)
- In vivo validation: availability of suitable in vivo models

2nd Prioritization Criteria

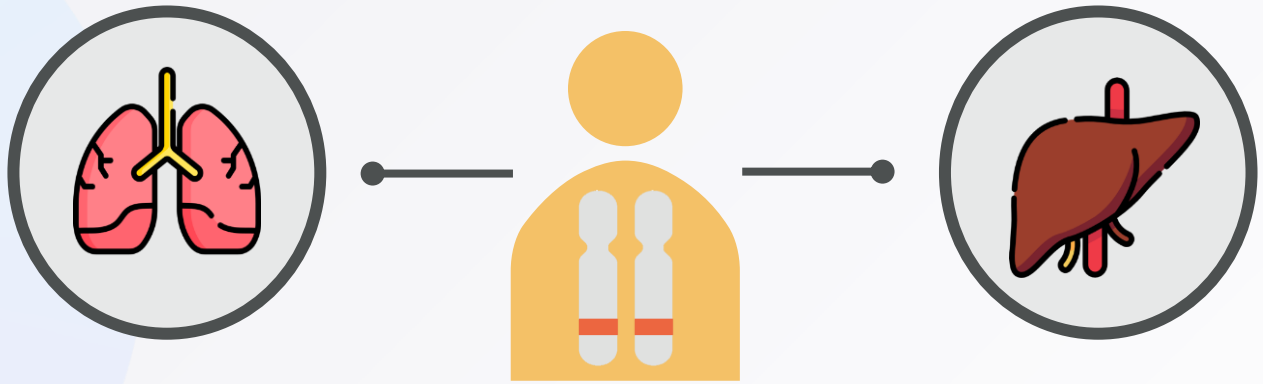
- Epidemiology: From Rare (1/100,000) to High (<1/2,000)
- Gene size: ORF ≤ 4,500 Kb

Indication Prioritization Final Step

Manual evaluation: Short-list of six diseases with strong fit for circVec identified

Alpha-1 antitrypsin deficiency (AATD) identified as opportunity for circVec

AATD is a major unmet medical need manifested in liver and lung



- Lack of functional AAT protein
- Emphysema and/or chronic bronchitis

- Toxic accumulation of mutant form of protein
- Cirrhosis

Moderate to severe AATD Diagnosed Patients

120K in EU

75K in US

Current treatment options

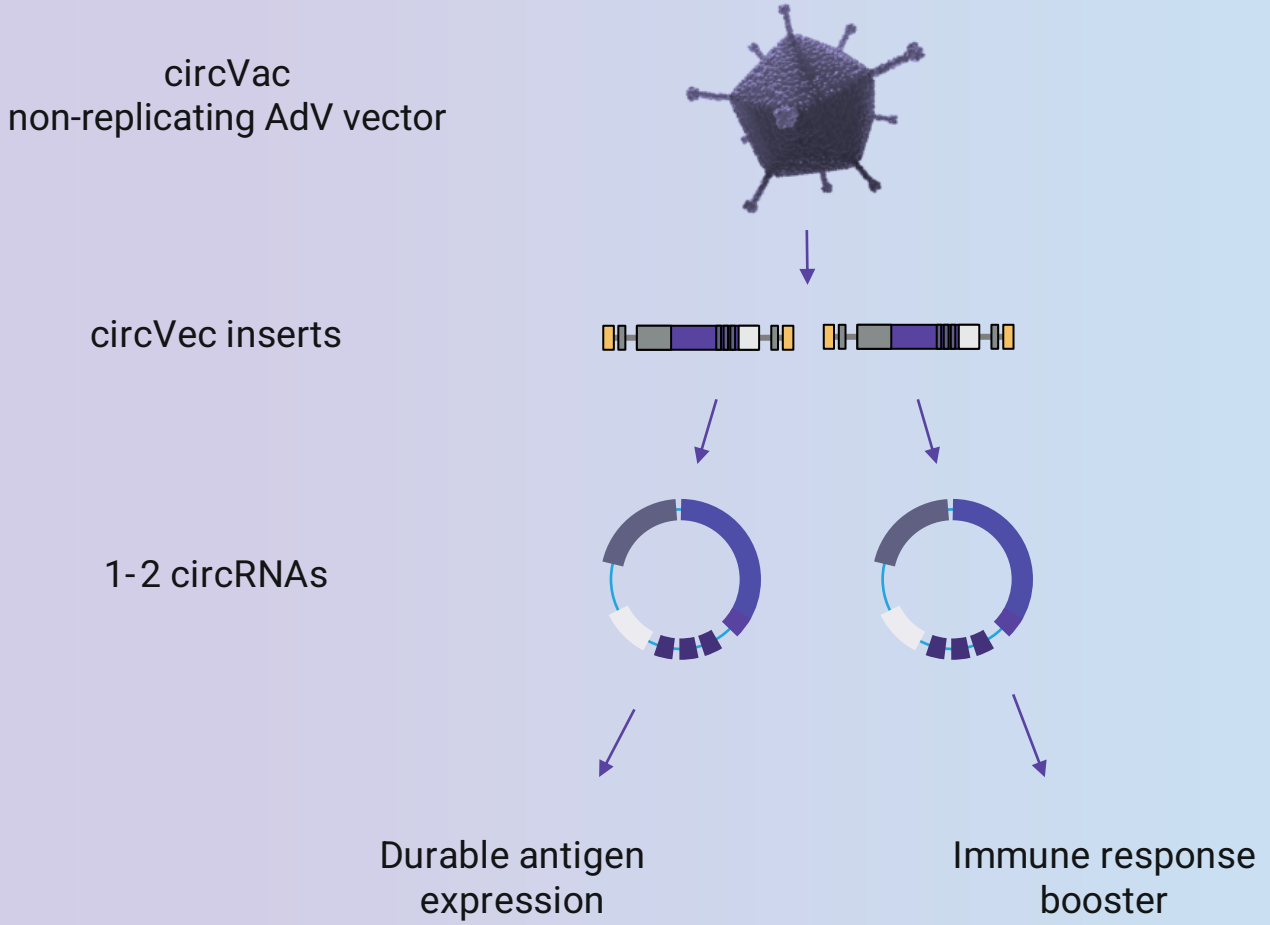
Lung-associated AATD

- Replacement therapy with an alpha-1 proteinase inhibitors
- Weekly IV infusions
- Bronchodilators and inhaled steroids used for mild symptoms

Liver-associated AATD

- No approved therapeutics
- Liver transplantation is the only treatment alternative in severe cases

circVac: Establishing proof-of-concept with aim to out-license for clinical development



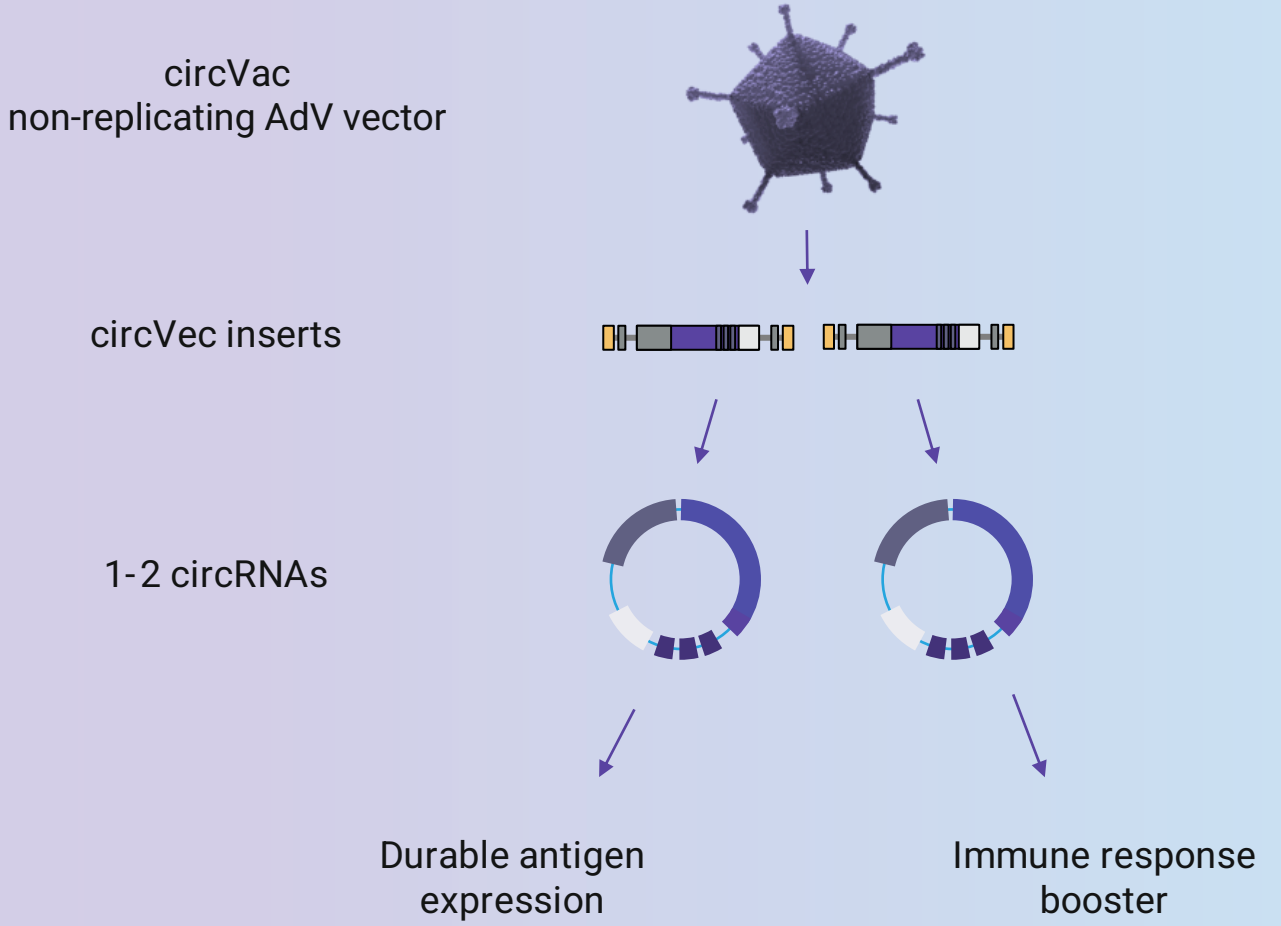
Development plan & target indication

- Major infectious diseases, incl. influenza, shingles, malaria
- Establish single dose vaccine concept
- Out-license technical concept for clinical development following pre-clinical PoC

Upcoming milestones

3Q´23:	First *in vivo* immunogenicity data
4Q´23:	COVID Spike circVac *in vivo* data
1H´24:	circVac v2.0 in vivo data

circVac: Washington University collaboration



Washington University in St. Louis

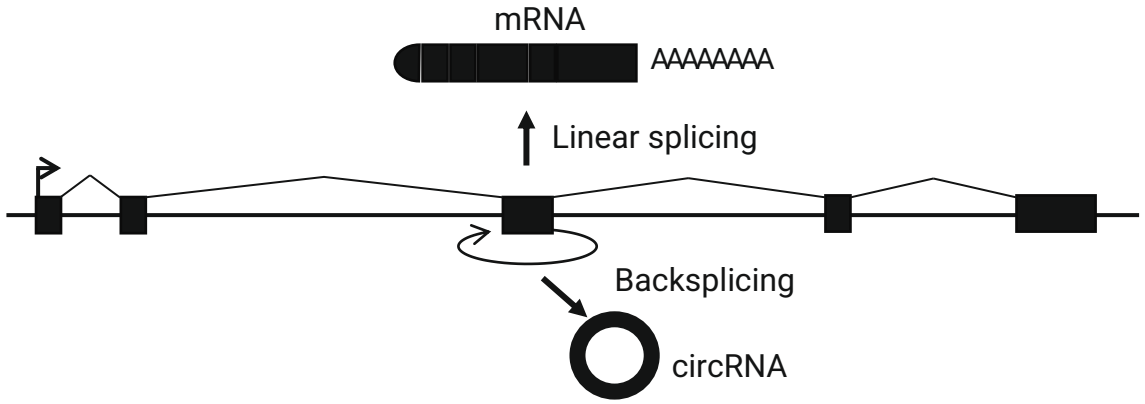
- *Collaboration to be initiated in October*
- *To be performed in the laboratory of professor David Curiel*
- *Test novel concept for circVac Flu vaccination*

2

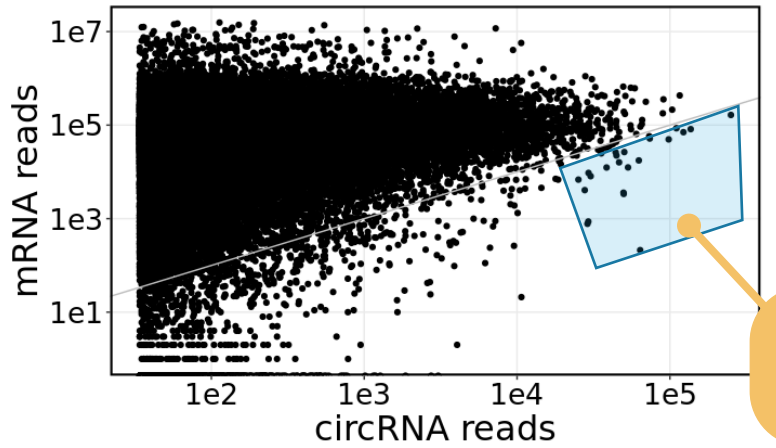
circVec data

3. Rare disease data
4. Summary & Next steps

circVec starting point is based on nature`s best design

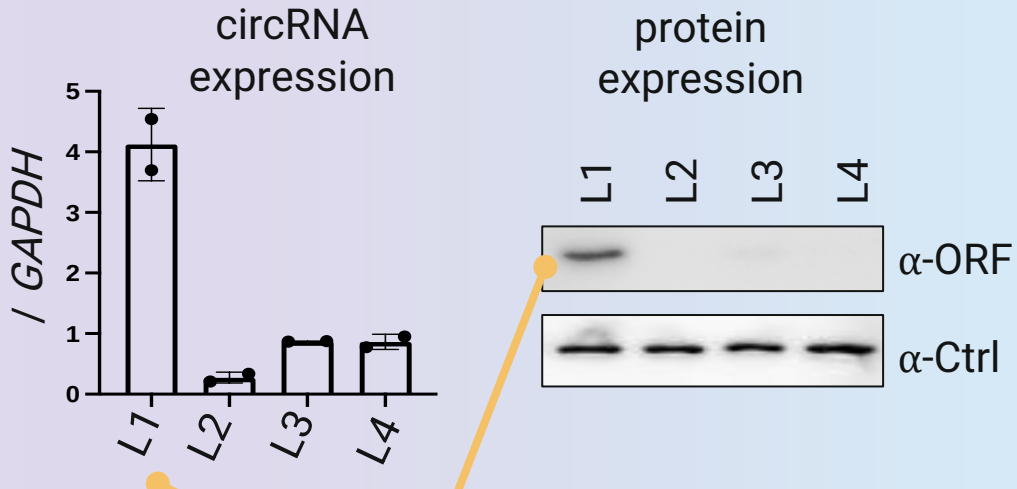


Expression of endogenous circRNA
NGS analysis of 300+ RNAseq datasets



Top natural circRNA loci

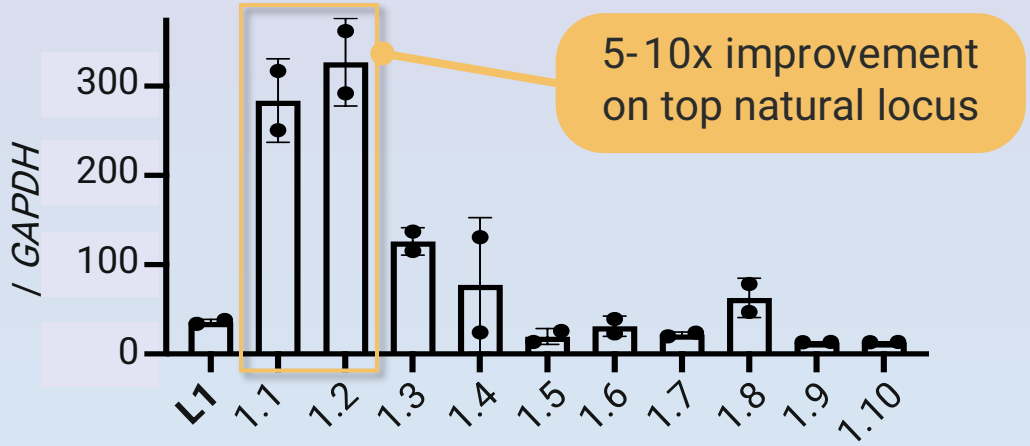
Screening of most effective circRNA-producing sequences invented by nature



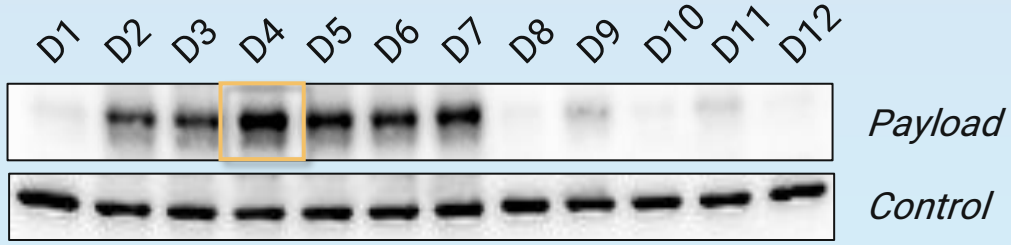
L1 design shows most efficient circRNA biogenesis and protein expression

circVec 1.0: Optimization of “Nature´s best design” for improved circRNA biogenesis and protein expression

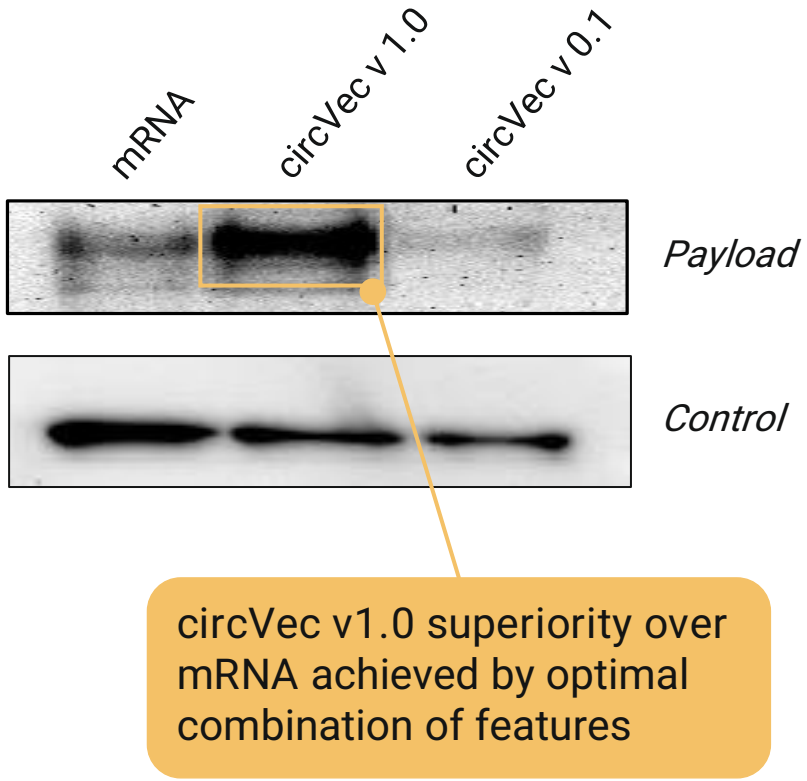
Design optimization for circRNA biogenesis
circRNA-specific RT-PCR; L1 = top wild-type



Design optimization for protein expression
Western blot, circRNA protein payload



circVec v1.0 design - outperforming mRNA
Western blot, protein expression



circVec 1.0 achieves 15x prolonged circRNA half-life and increased protein expression vs. mRNA *in vitro*

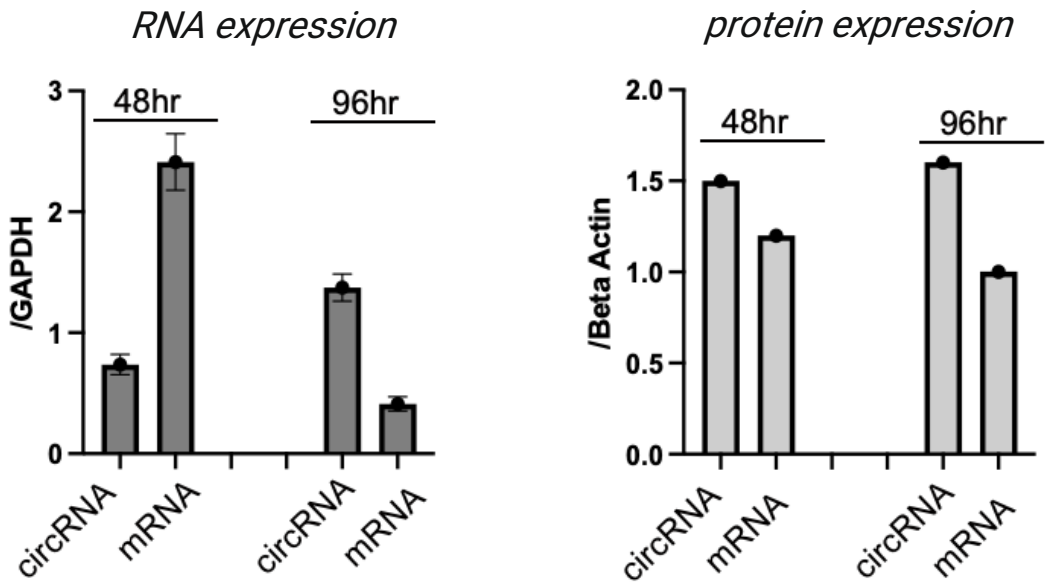
circVec v1.0 RNA stability
RT-qPCR, nascent vs. total RNA

135h vs. **9h**

circRNA mRNA

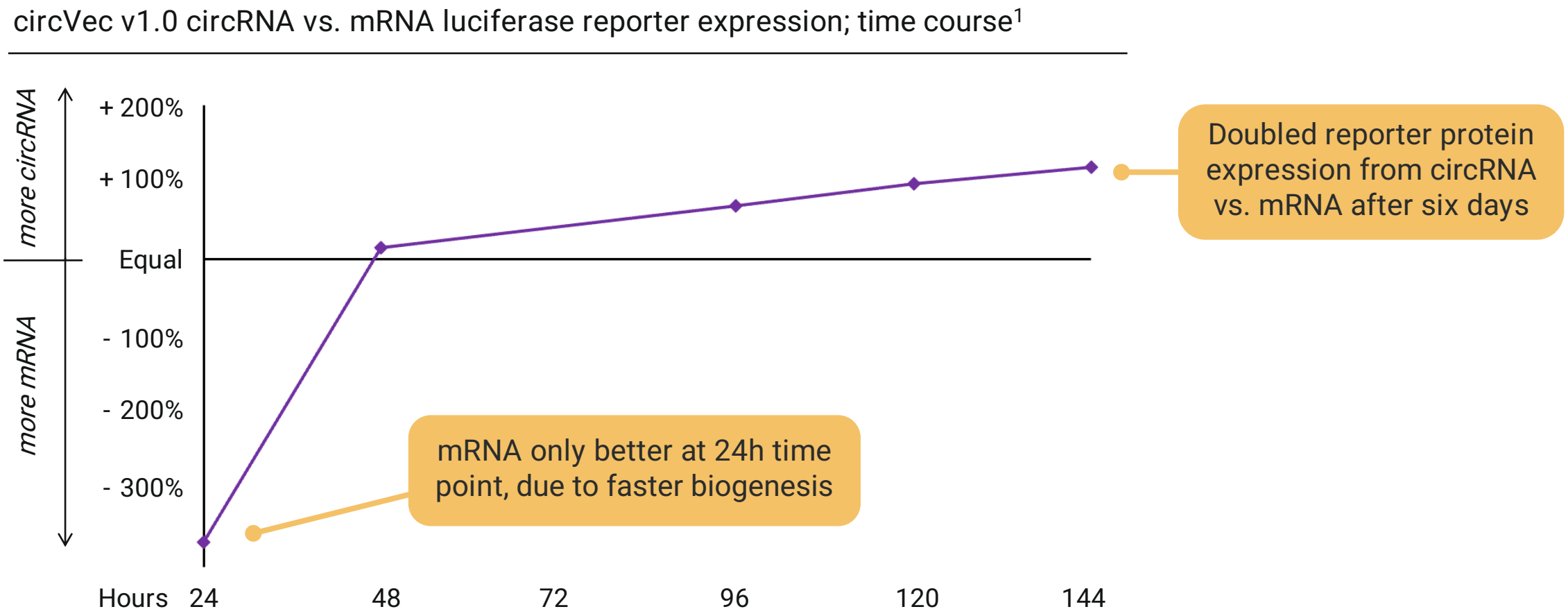
15 x

Accumulation of circVec v1.0 circRNA and protein payload over time, RT-PCR and Western blot



circRNA outperforms mRNA in vitro – comparative *in vivo* experiments ongoing with circVec v1.0

Doubled circVec 1.0 protein expression from circRNA vs. mRNA vectors after six days

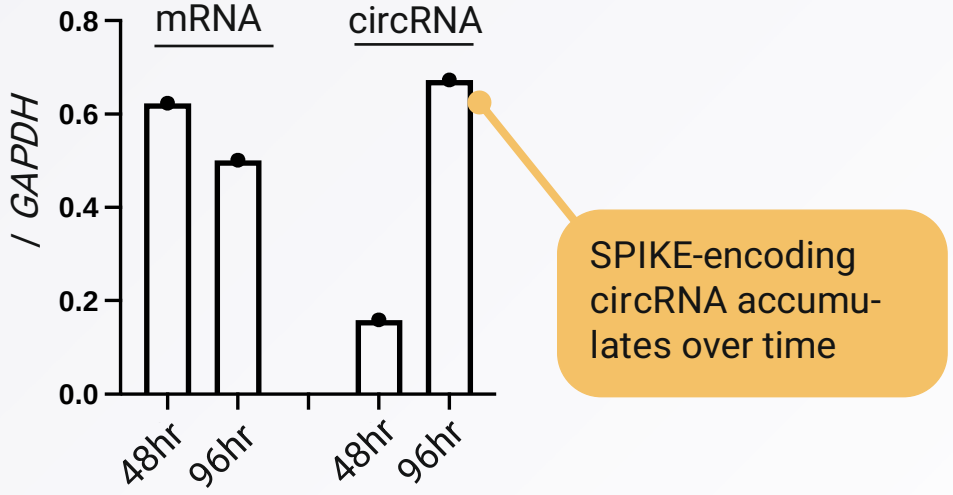


1 Renilla vs. Firefly luciferase relative levels

Translating into vaccines: durable circVac 1.0 expression of COVID Spike protein

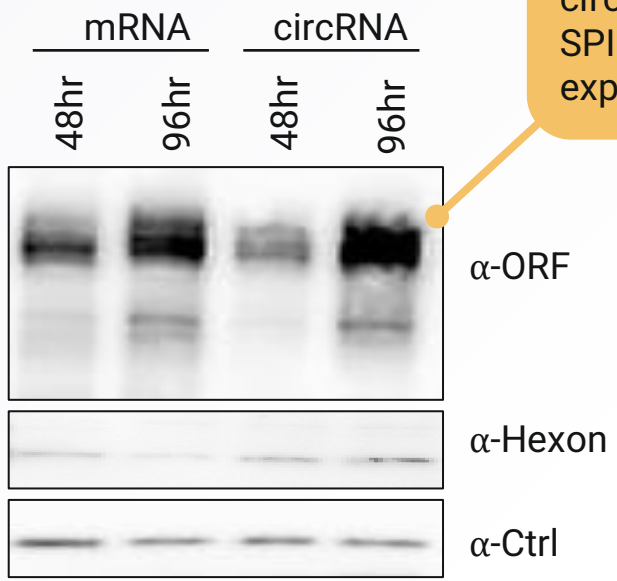
SPIKE expression from circVac, RNA and protein level

RNA expression



SPIKE-encoding circRNA accumulates over time

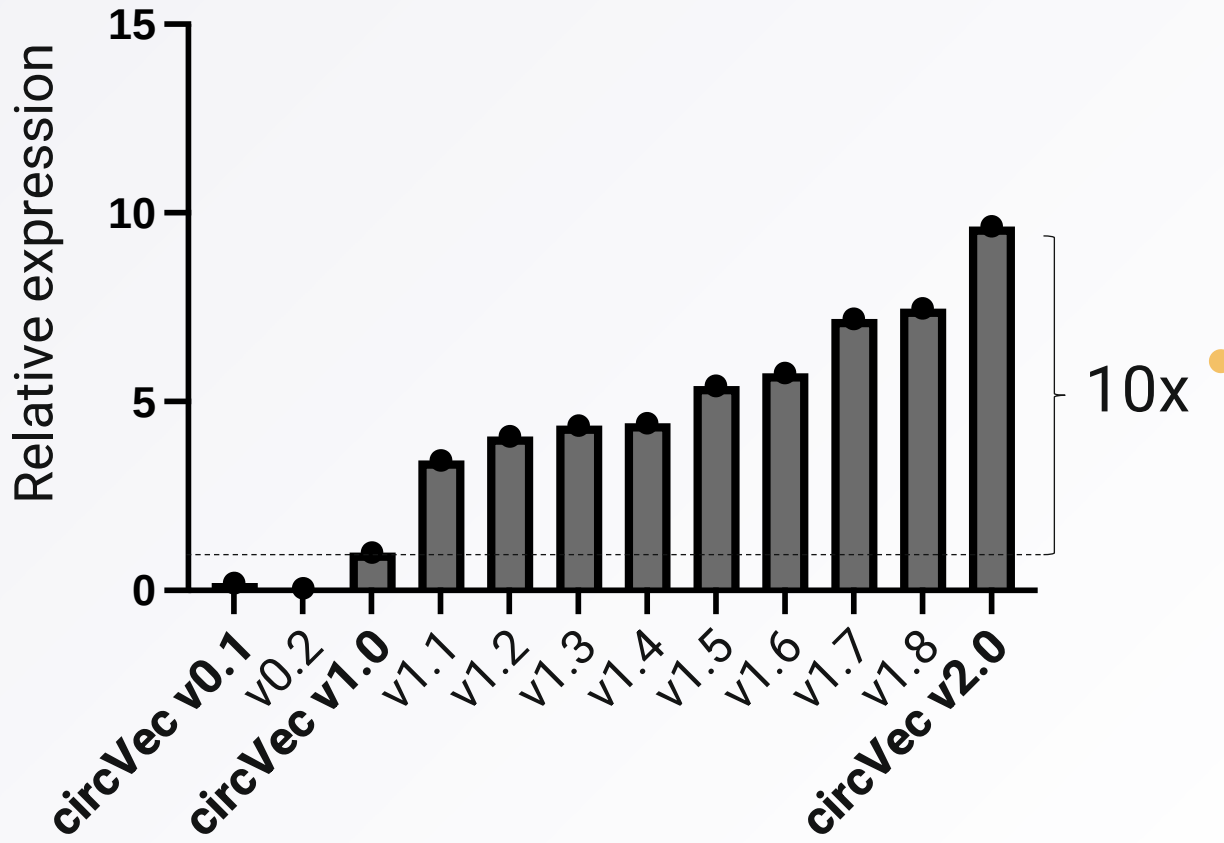
Protein expression



circRNA > mRNA SPIKE protein expression @ 96hr

circVec 2.0: Design optimization has resulted in >10x further improvement in protein expression

circVec sequence optimization, protein expression level

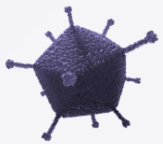


circVec v2.0 achieves 3-10x[1] higher protein yield than v1.0 design

1 Level of improvement in range of 3-10x depending on cell type

Early *in vivo* data confirm circVec 1.0 functionality

- circVec v1.0 circRNA biogenesis and protein expression confirmed for DNA vector in immunodeficient mice



- circVec v1.0 circRNA biogenesis and protein expression confirmed in solid tumors from replicating Adenovirus (AdV) circVec vector

- circVac v1.0 immunogenicity confirmed for non-replicating AdV vector in normal healthy mice

Further optimization of experimental set-up required for comparative analysis of activity

Constructs being generated and *in vivo* experiments planned for new circVec 2.0 design

3

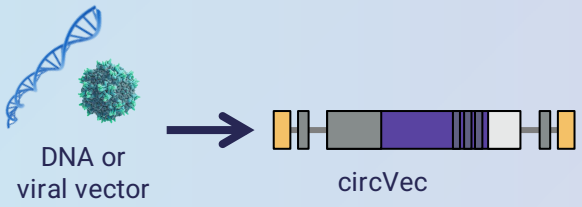
Rare disease data

4. Summary & Next steps

circVec offers a ”Remove-and-Replace” option for AATD

Depleting mutant form and replenishing functional protein by circVec

- *reverses toxic protein accumulation in liver and restores normal function in lung*



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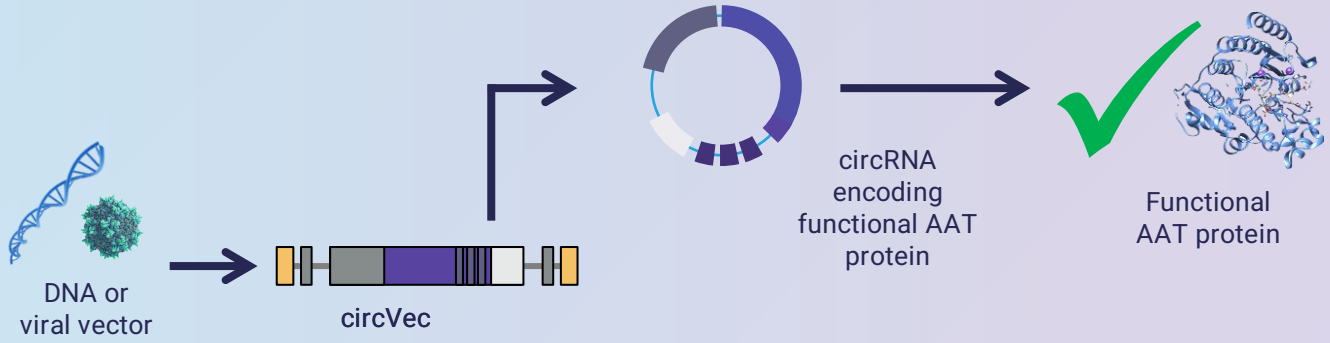

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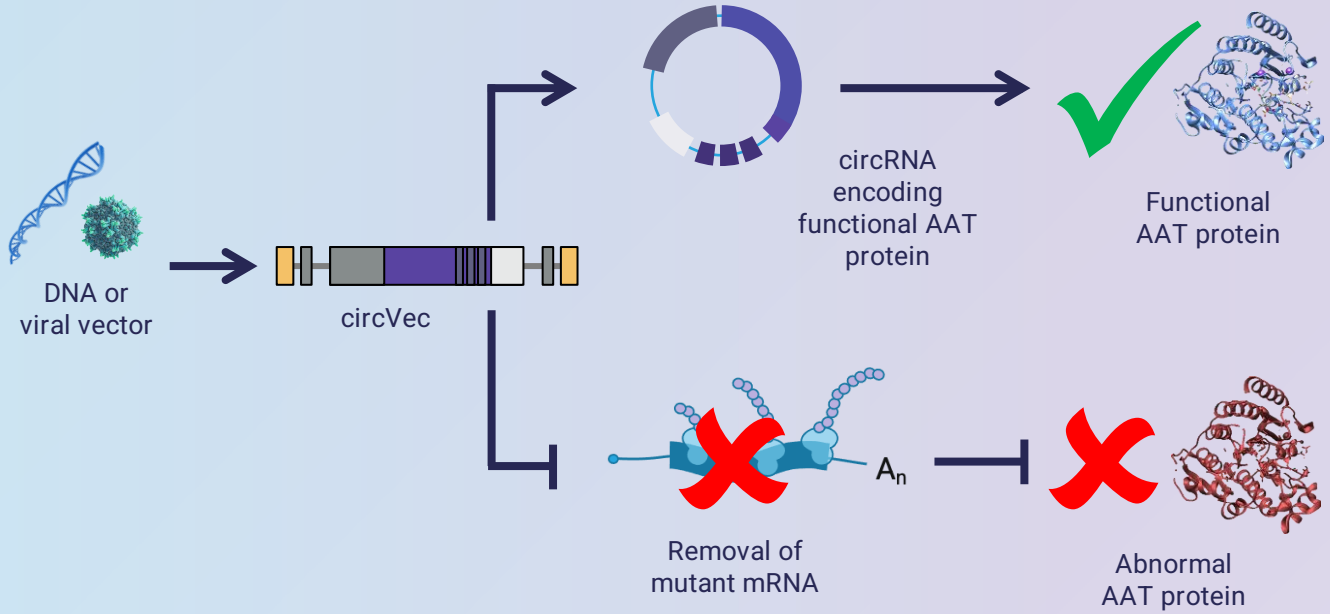

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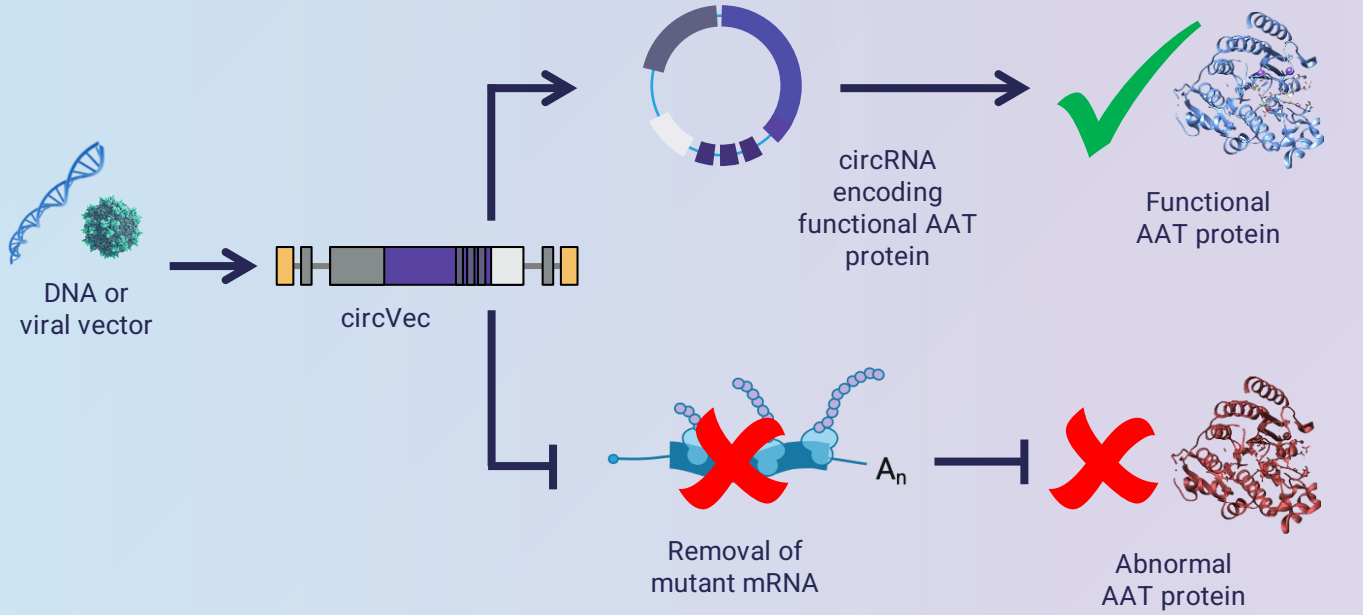
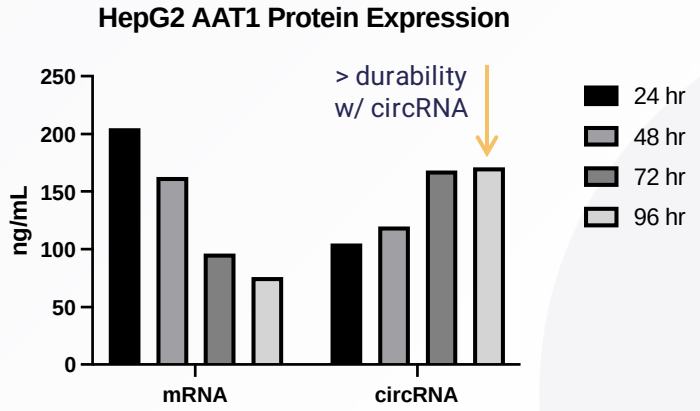


circVec AAT expression in liver cells



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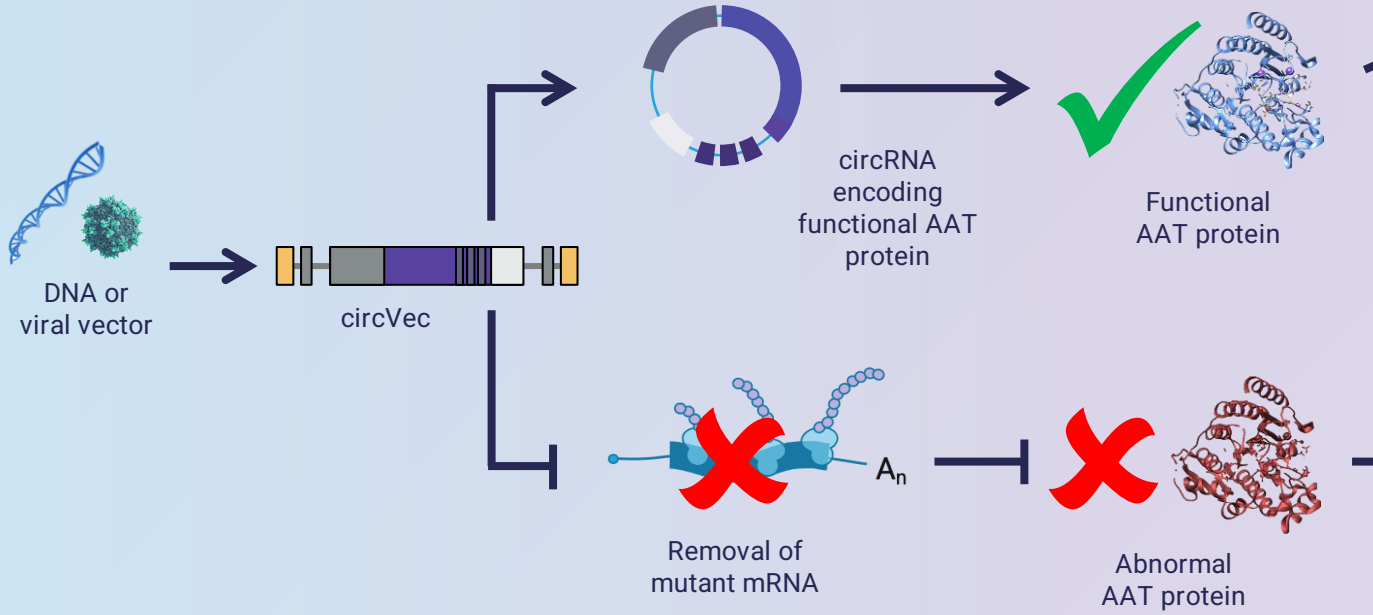

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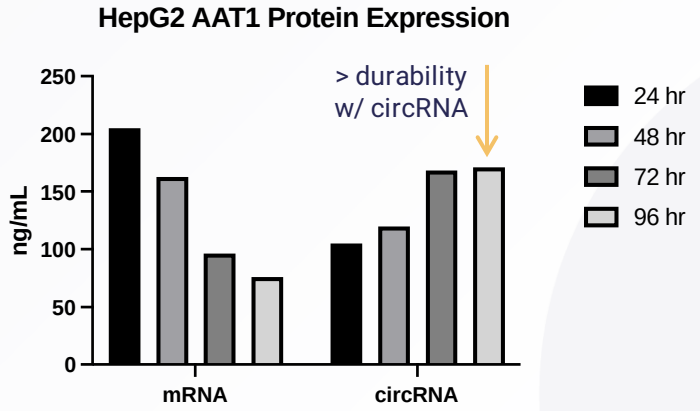
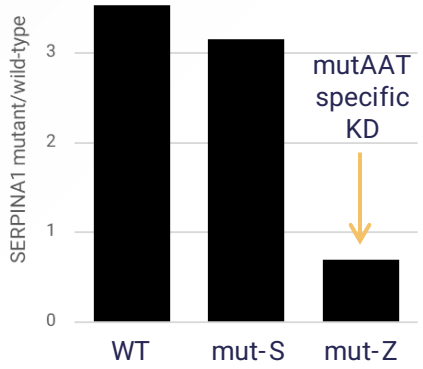


mutAAT knock-down



4

Summary & Next steps

circVec data summary: broad technical proof-of-concept established

circRNA biogenesis

- 10x biogenesis rate vs. “nature´s best design”
- 15x extended half-life vs. mRNA *in vitro*
- circVec circRNA biogenesis confirmed *in vivo*

- *Next step: Optimize biogenesis in vivo*

Vector functionality

- DNA and virus-based circRNA biogenesis
- Optimized circVec 2.0 design established
- Ability to generate up to at least 5kb circRNAs

- *Next step: Test circVec 2.0 in multiple vector types in vitro and in vivo*

Protein expression

- 3-5x enhanced protein expression vs. mRNA
- Validated for multiple protein types, incl. AAT
- Protein expression confirmed *in vivo*

- *Next step: Validate expression and durability of circVec 2.0 in vivo*

Regulatory functions

- miRNA sponging activity confirmed
- Additional KD functionality established
- “Hi-jacking” of host cell protein expression

- *Next step: Combine functionalities in vitro*

Characterization of therapeutically relevant vector systems *in vitro* 2H'23, *in vivo* 1H'24

2023

1	AAV	Oct-Dec Evaluation of circRNA *in vitro* expression profile from AAV virus compared to conventional mRNA-based AAVs
2	DNA v1	Oct-Dec Characterization of DNA format 1 as vector for circRNA expression
3	DNA v2	Nov-Jan Characterization of DNA format 2 as vector for circRNA expression

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In vivo experimental read-outs 2H'23

circRNA vs. mRNA circVec DNA vectors

2023

1	Vaccine efficacy	Oct-Dec Evaluation of immunogenicity and T-cell responses in mice immunized with circVac v1.0 expressing COVID Spike protein
2	Reporter expression durability	Nov-Dec Characterization of circVec v2.0 Firefly luciferase reporter expression level and durability
3	AAT expression durability	Dec-Jan Characterization of circVec v2.0 AAT protein expression level and durability

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Circio has a unique position in the circRNA field

- Circio is the only significant player in the DNA-format circRNA space

- Enhanced durability and protein expression from circRNA is expected to translate into lower dosing of DNA-format applications, which may solve both potency, toxicity and cost challenges facing current ”gold-standard” gene therapy

- Vector-expressed circRNA has the potential to become the preferred format for any DNA-based therapeutic in the future
  - *Just as synthetic circRNA is expected to become the preferred format for long RNA-based therapeutics in the future*